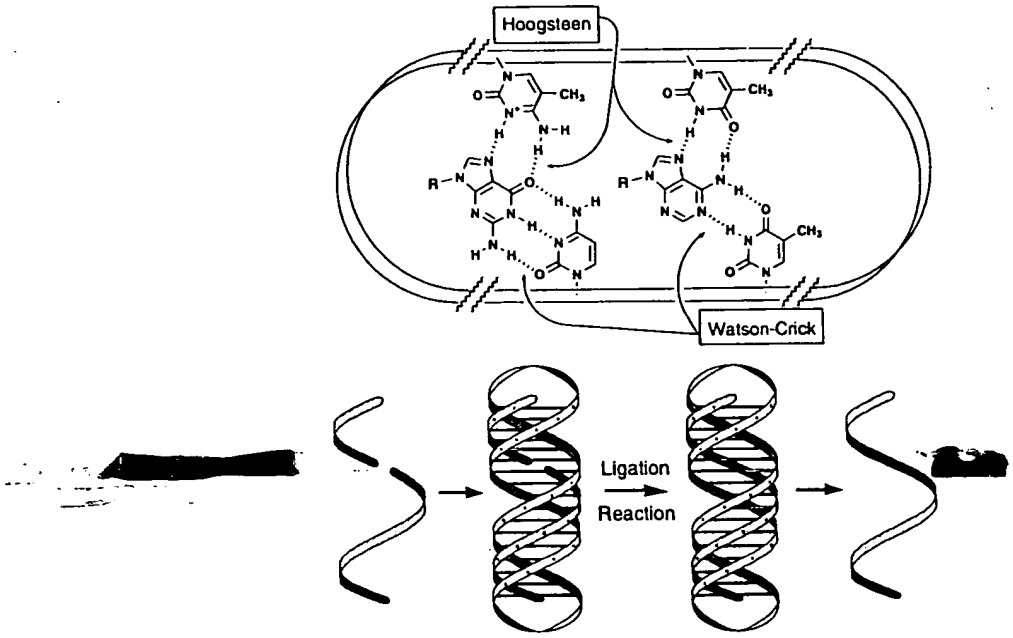
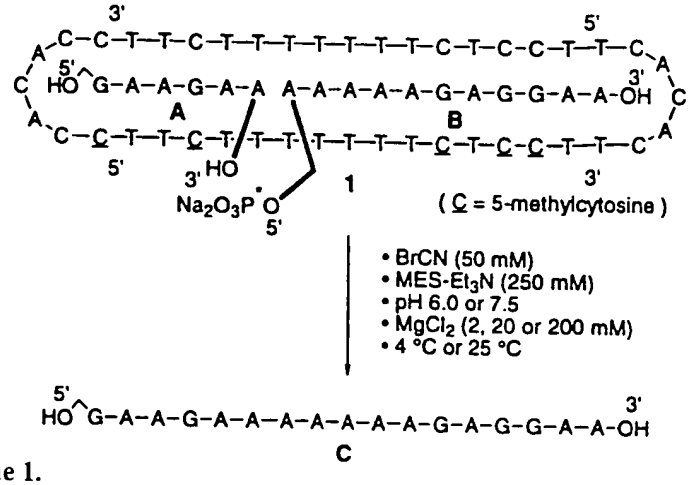


Figure 1. Top: triplex resulting from homopurine ligation fragments bound to pyrimidine bases of the circular DNA template (**R** represents backbone of ligating fragments). Note the use of 5-methylcytidine on the Hoogsteen side of the circular template. Bottom: ribbon graphic of a ligation reaction of two ODNs directed by a circular DNA template.


3'
5'
A
C–C–T–T–C–T–T–T–T–T–T–T–C–T–C–C–T–T–C
C
5'
HO
G–A–A–G–A–A
A–A–A–A–A–G–A–G–G–A–A–OH
3'
A
C
A
A
B
C–C–T–T–C–T–T–T–T–T–T–T–C–T–C–C–T–T–C
A
5'
3'
HO
3'
1
Na2O3P
O
5'
(C = 5-methylcytosine)
• BrCN (50 mM)
• MES-Et3N (250 mM)
• pH 6.0 or 7.5
• MgCl2 (2, 20 or 200 mM)
• 4 °C or 25 °C
5'
HO
G–A–A–G–A–A–A–A–A–A–A–G–A–G–G–A–A–OH
3'
C

Scheme 1.

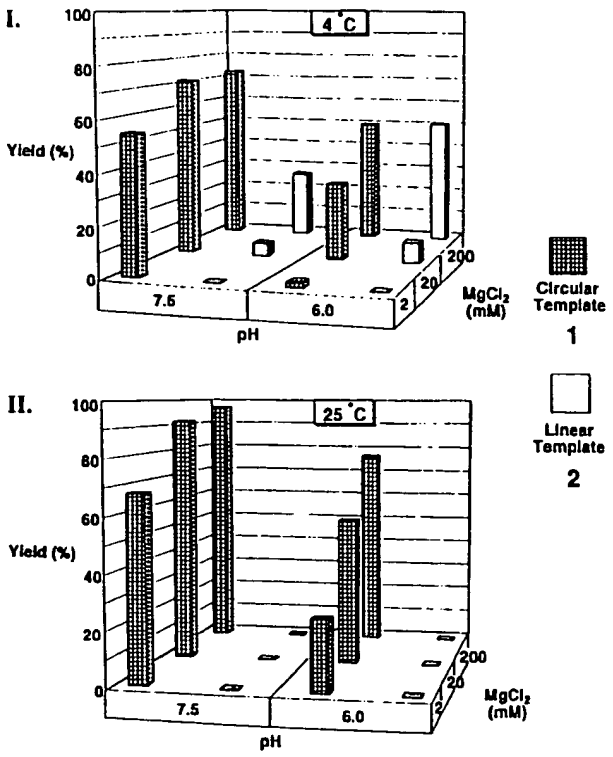
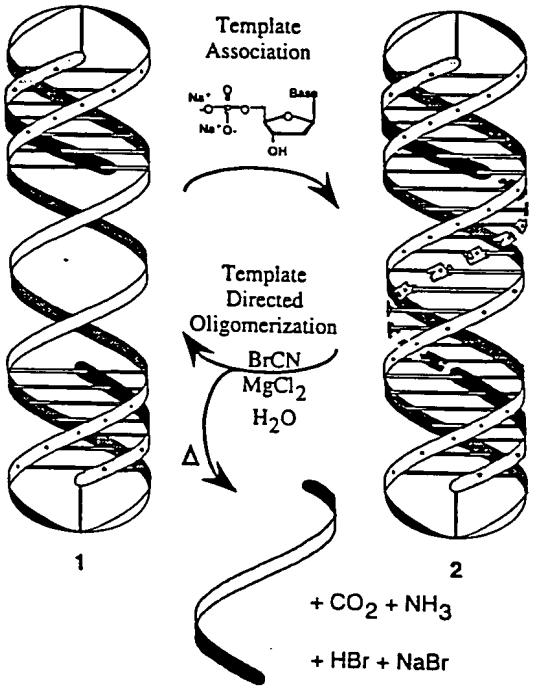


Figure 2. 3-D bar graphs showing the yield (%) of ligation product C. Graph I shows ligation results at 4°C, pH 7.5 and 6.0 with $MgCl_2$ concentrations of 2, 20 and 200 mM. Graph II shows the same ligation reactions run at 25°C. Data for these graphs was obtained at a reaction time of 3 h. All reactions were reproduced at least twice to afford a % yield error of ±3.

Figure 3



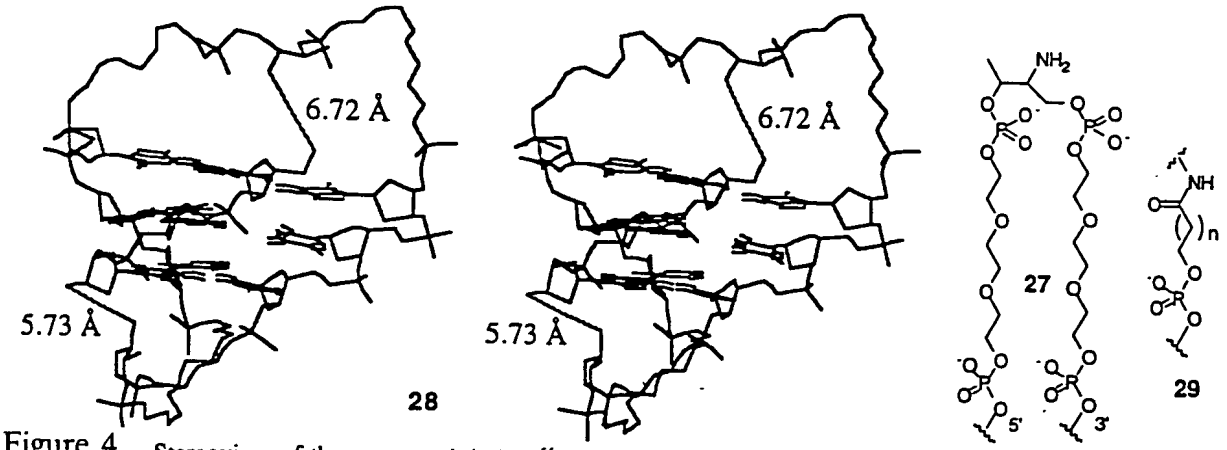
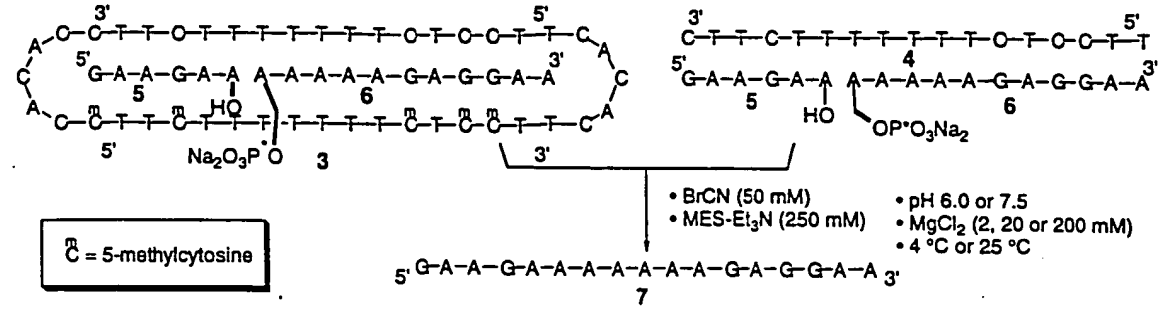
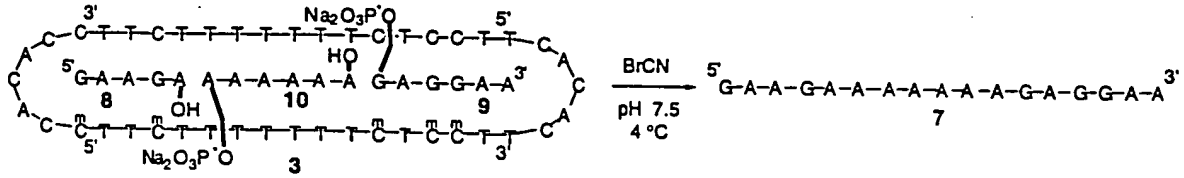


Figure 4 . Stereoview of the energy minimized[56] circular DNA template (**28**) with looped linker **27** attached at both ends of the truncated triplex. Based on structure **28**, the initial template-primer linker for investigation will be **29** (n = 2). Based on an MM2 minimized structure, **29** will span a distance up to 7 Å.

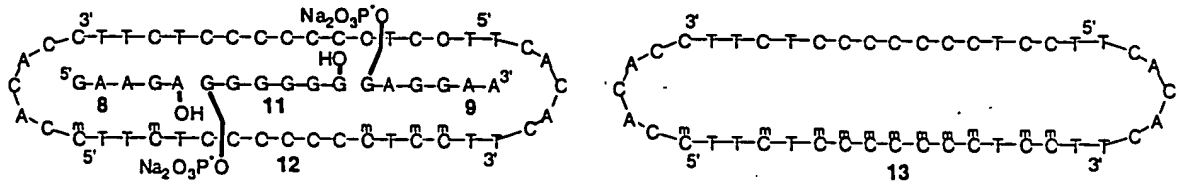
Scheme 2.



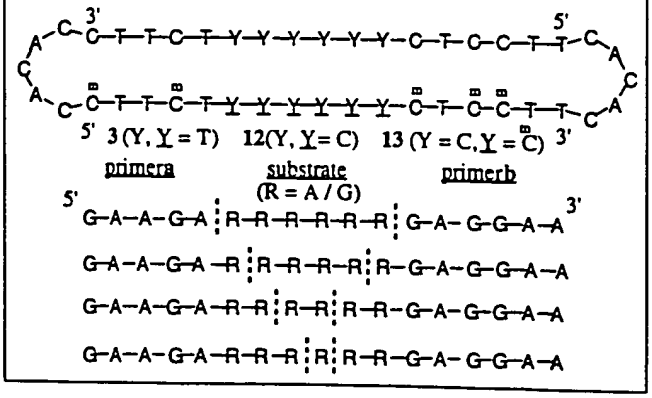
Scheme 3.



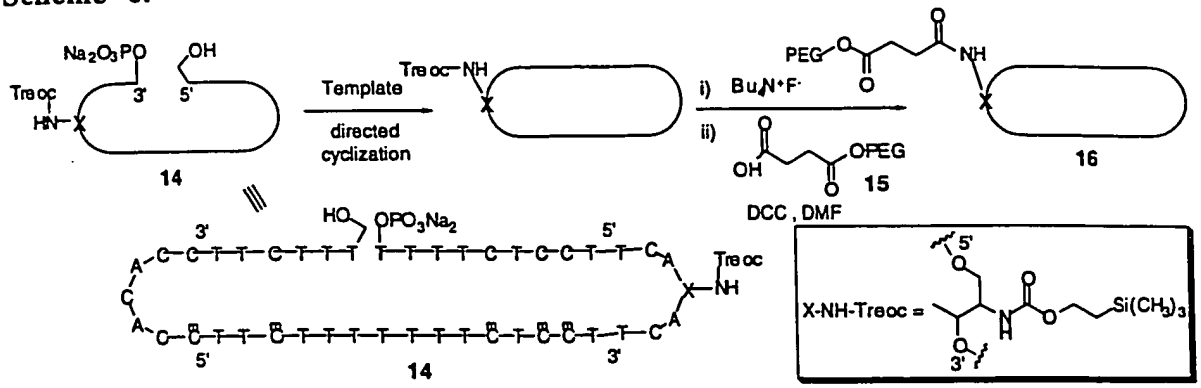
Scheme 4.



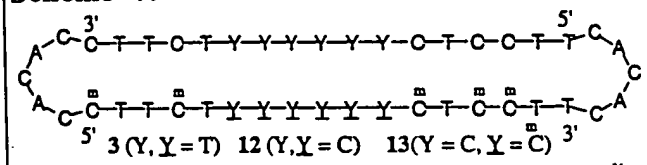
Scheme 5.



Scheme 6.

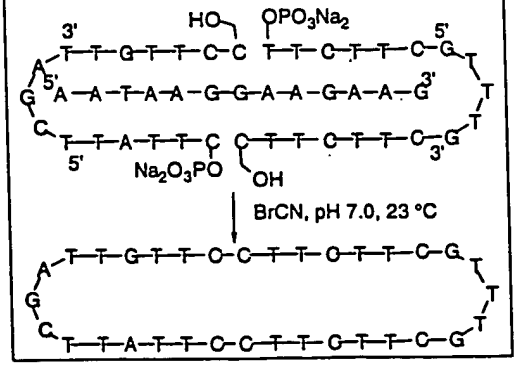


Scheme 7.

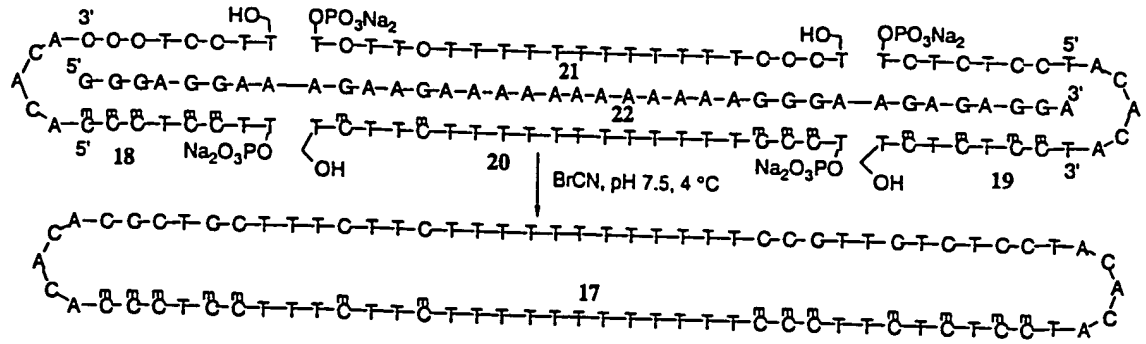


5'	5	substrate	9	3'	ligations
	G-A-A-G-A	R-R-R-R-R-R	G-A-G-G-A-A		2
	G-A-A-G-A	R-R-R:R-R-R	G-A-G-G-A-A		3
	G-A-A-G-A	R-R:R-R:R-R	G-A-G-G-A-A		4
	G-A-A-G-A	R:R:R:R:R:R	G-A-G-G-A-A		oligomerization

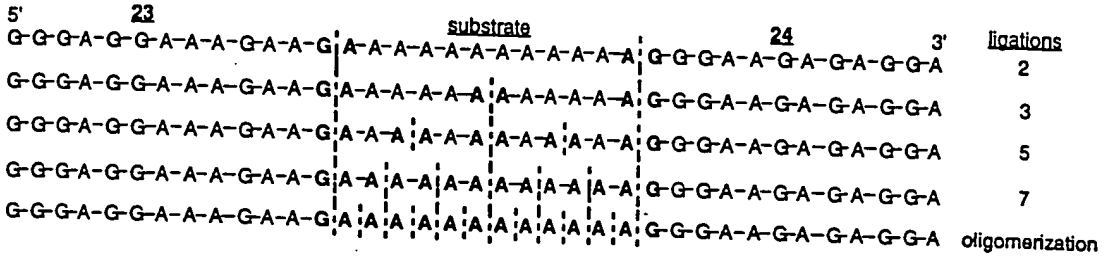
Scheme 8.



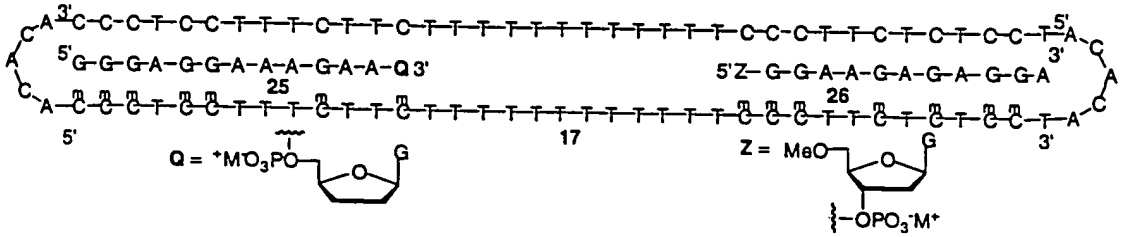
Scheme 9.



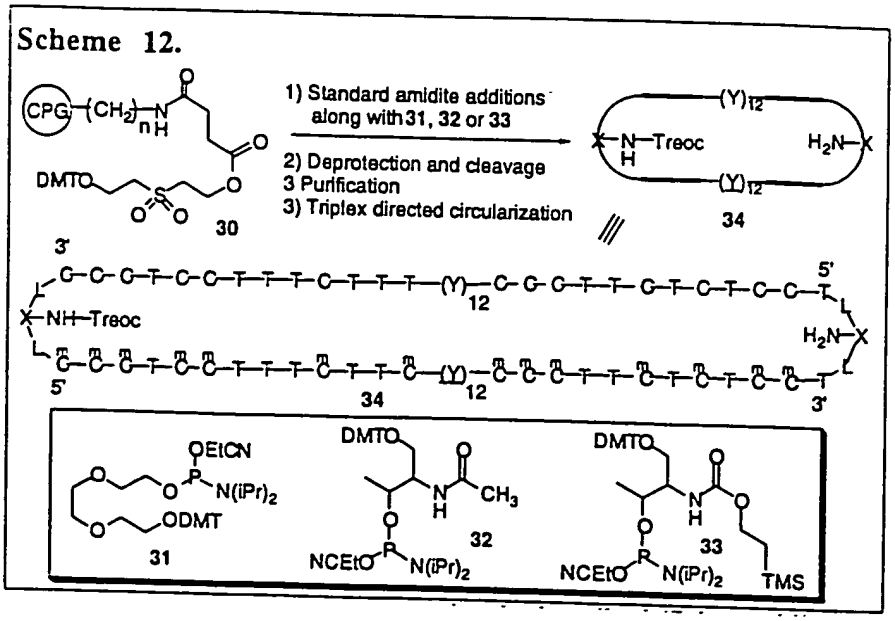
Scheme 10.



Scheme 11.



Scheme 12.



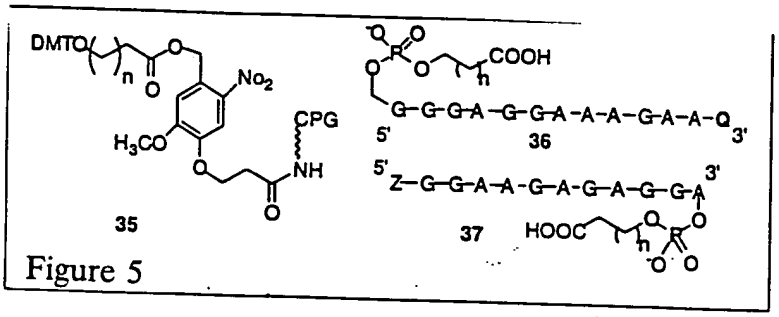


Figure 5

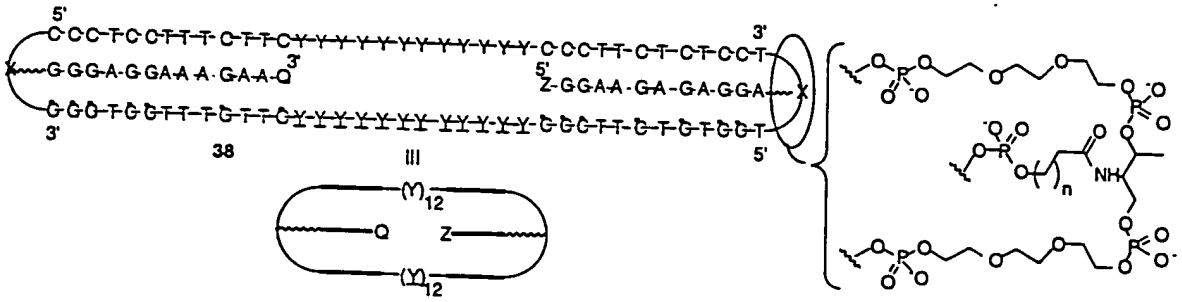
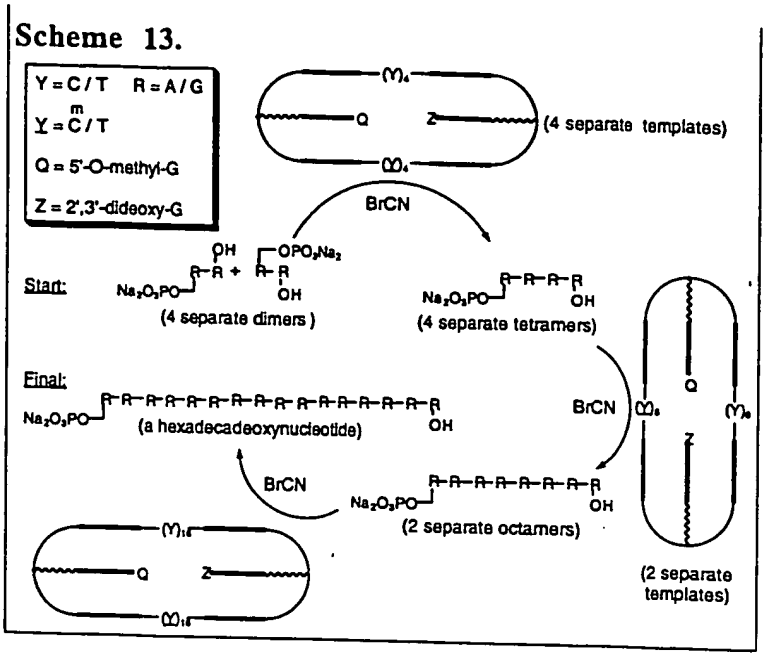
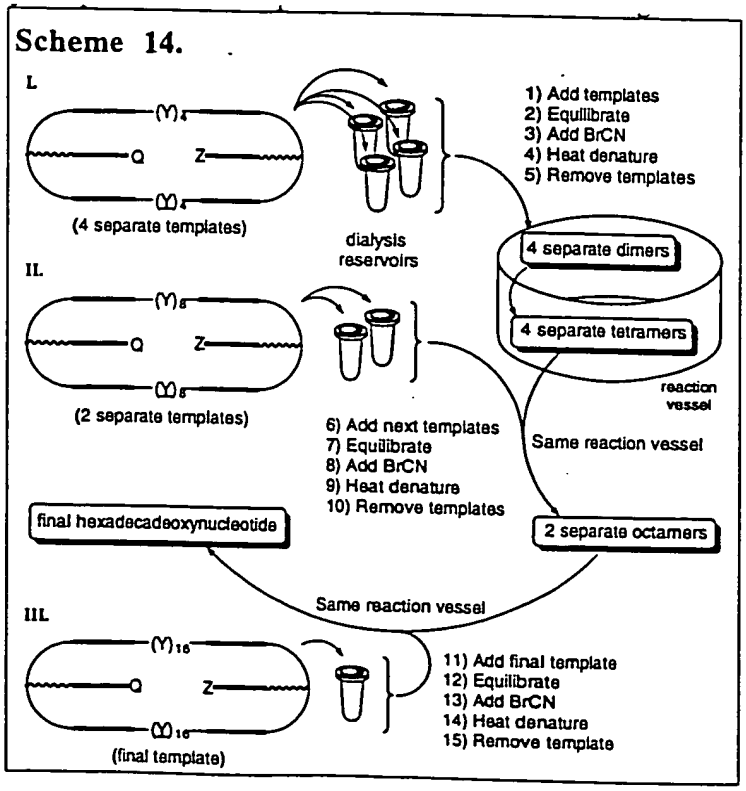


Figure 6 . Primer attached circular DNA template. See Scheme 11 for Q and Z designation.

Scheme 13.



Scheme 14.



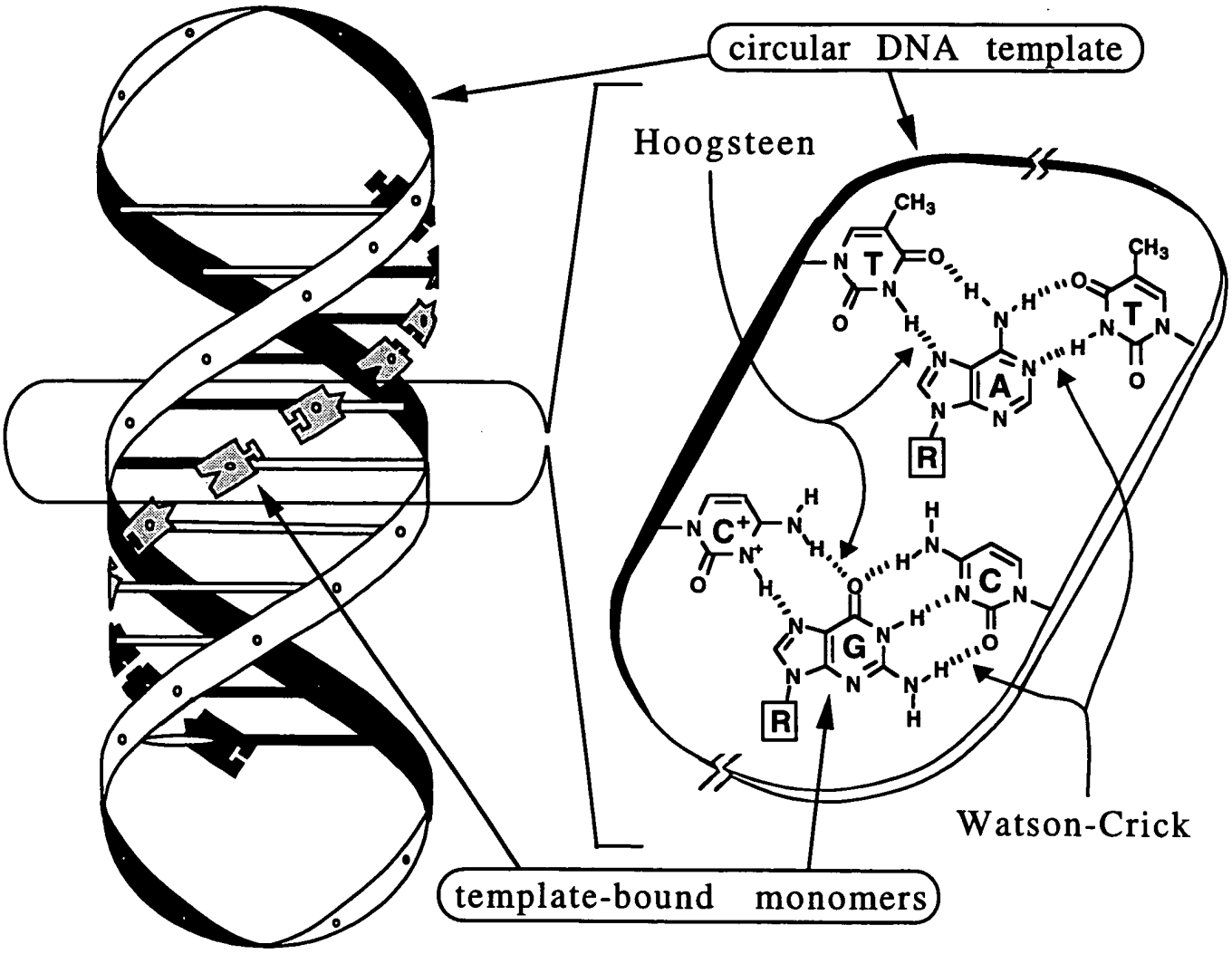


FIG. 7 Ribbon graphic of a circular DNA template with bound monomer nucleobase derivatives and illustration of the template-substrate triplets (**R** represents reacting substrate for oligomerization).

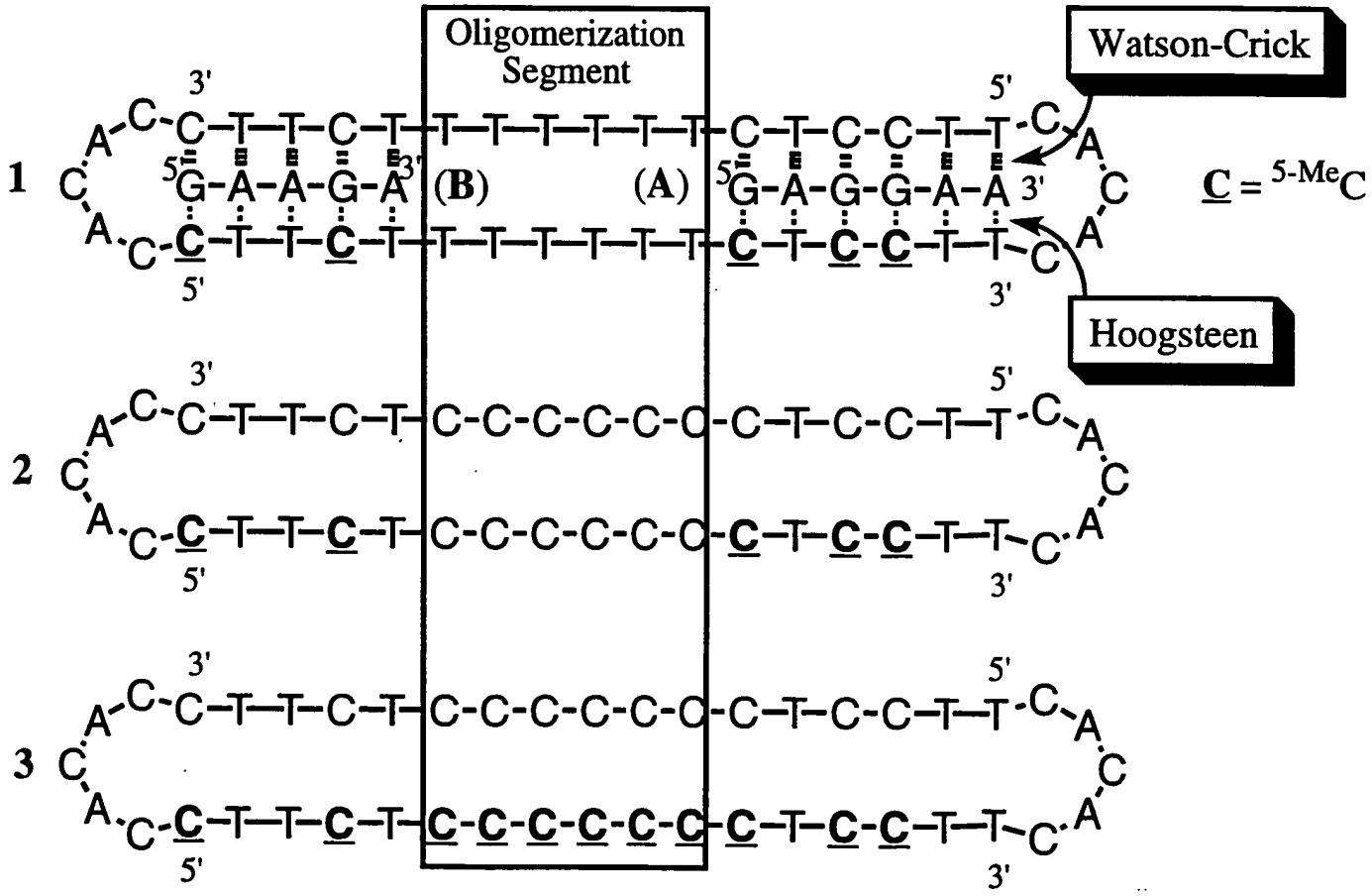
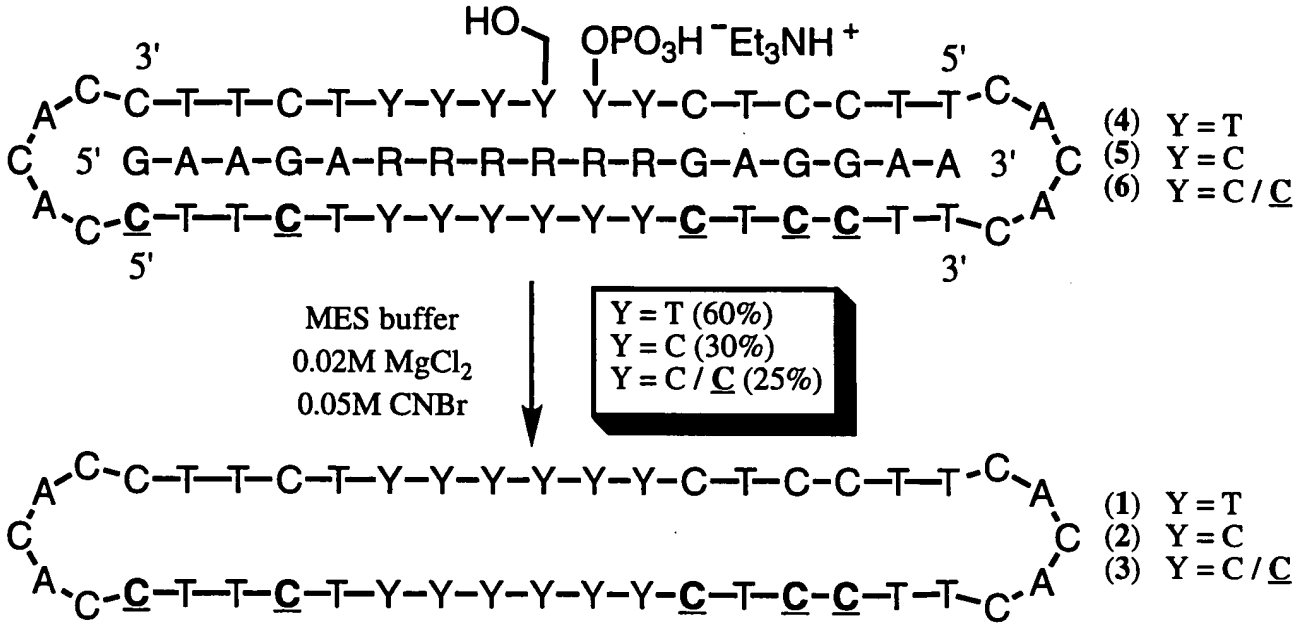


FIG. 8 Three Circular DNA templates (**1**, **2**, and **3**) and their respective primers (**A** and **B**) for directed ligation and oligomerization experiments. The C designates 5-methyl-cytidine.

Scheme **15**


HO
OPO3H⁻Et3NH⁺
3'
5'
A C C–T–T–C–T–Y–Y–Y–Y Y–Y–C–T–C–C–T–T C A
C 5' G–A–A–G–A–R–R–R–R–R–R–G–A–G–G–A–A 3' C
A C C–T–T–C–T–Y–Y–Y–Y–Y–Y–C–T–C–C–T–T C A
5'
3'
(4) Y = T
(5) Y = C
(6) Y = C / C
MES buffer
0.02M MgCl2
0.05M CNBr
Y = T (60%)
Y = C (30%)
Y = C / C (25%)
A C C–T–T–C–T–Y–Y–Y–Y–Y–Y–C–T–C–C–T–T C A
C
A C C–T–T–C–T–Y–Y–Y–Y–Y–Y–C–T–C–C–T–T C A
(1) Y = T
(2) Y = C
(3) Y = C / C

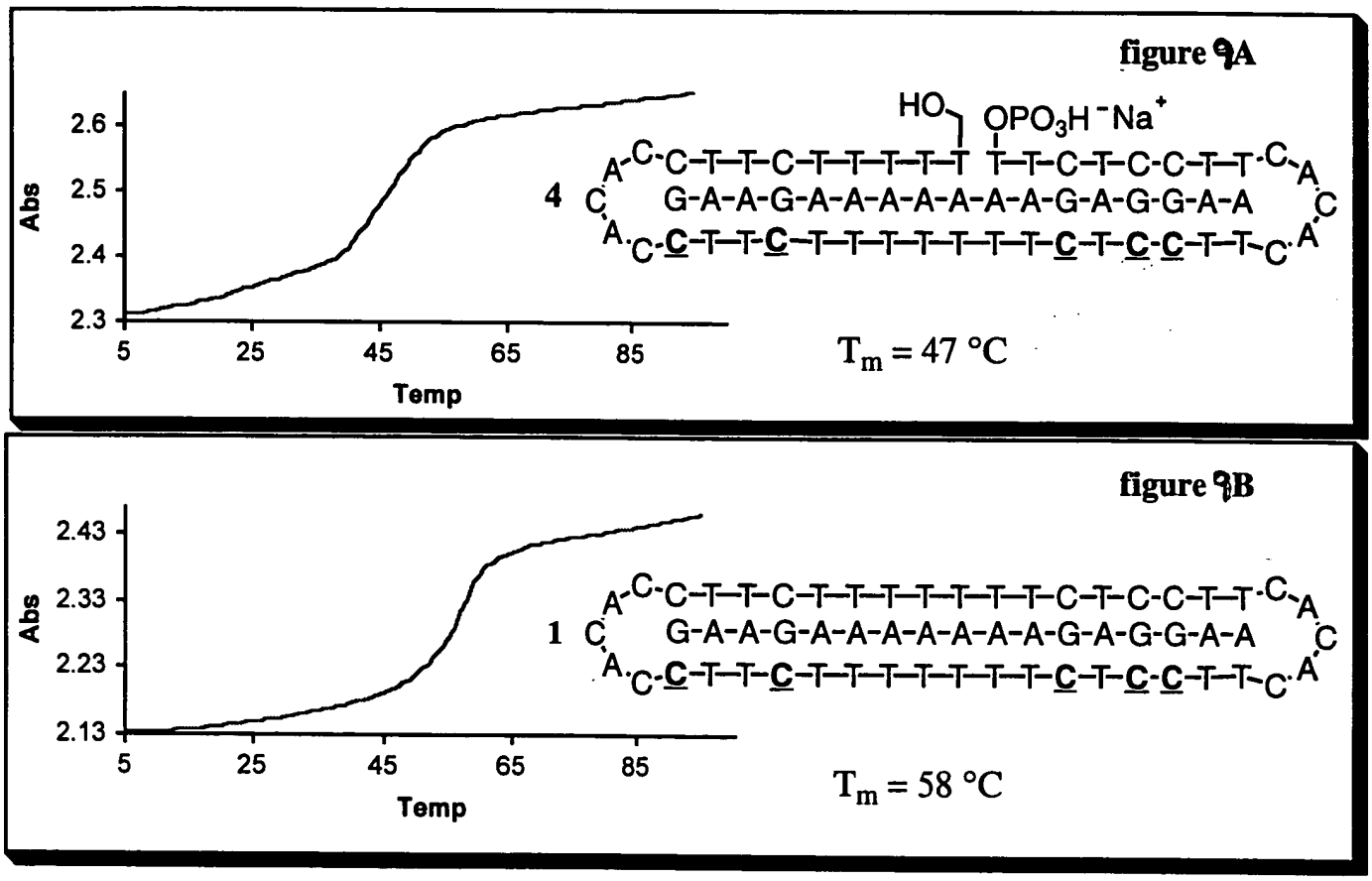


FIG. 9 Melting analysis of 4 with the purine-rich oligonucleotide (**3A**) and circularized template **1** with the same oligonucleotide (**3B**).

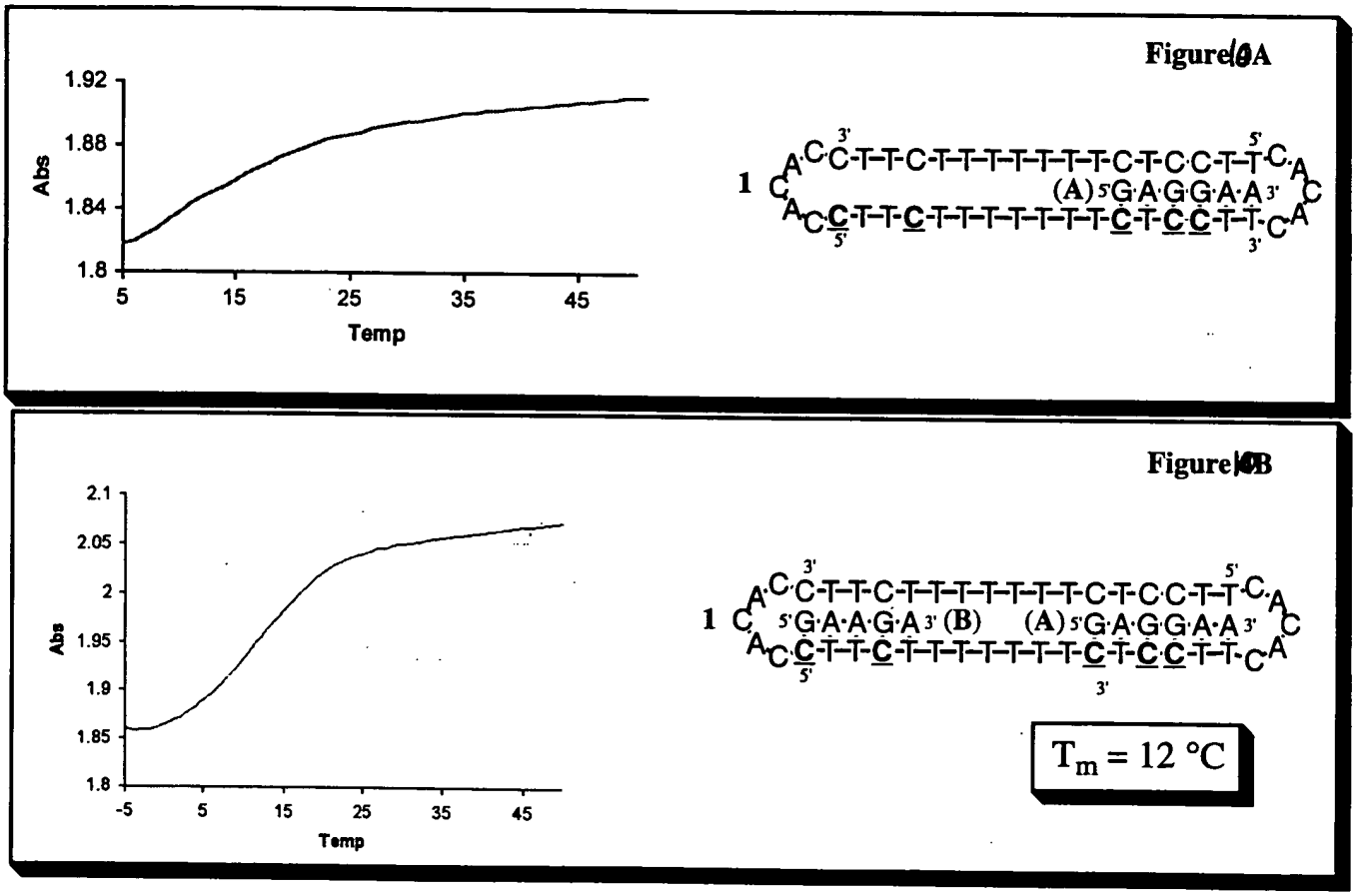


FIG. 10 Melting analysis of circular template **1** with primer **A** (fig 10A) and primers **A** and **B** (fig 10B).

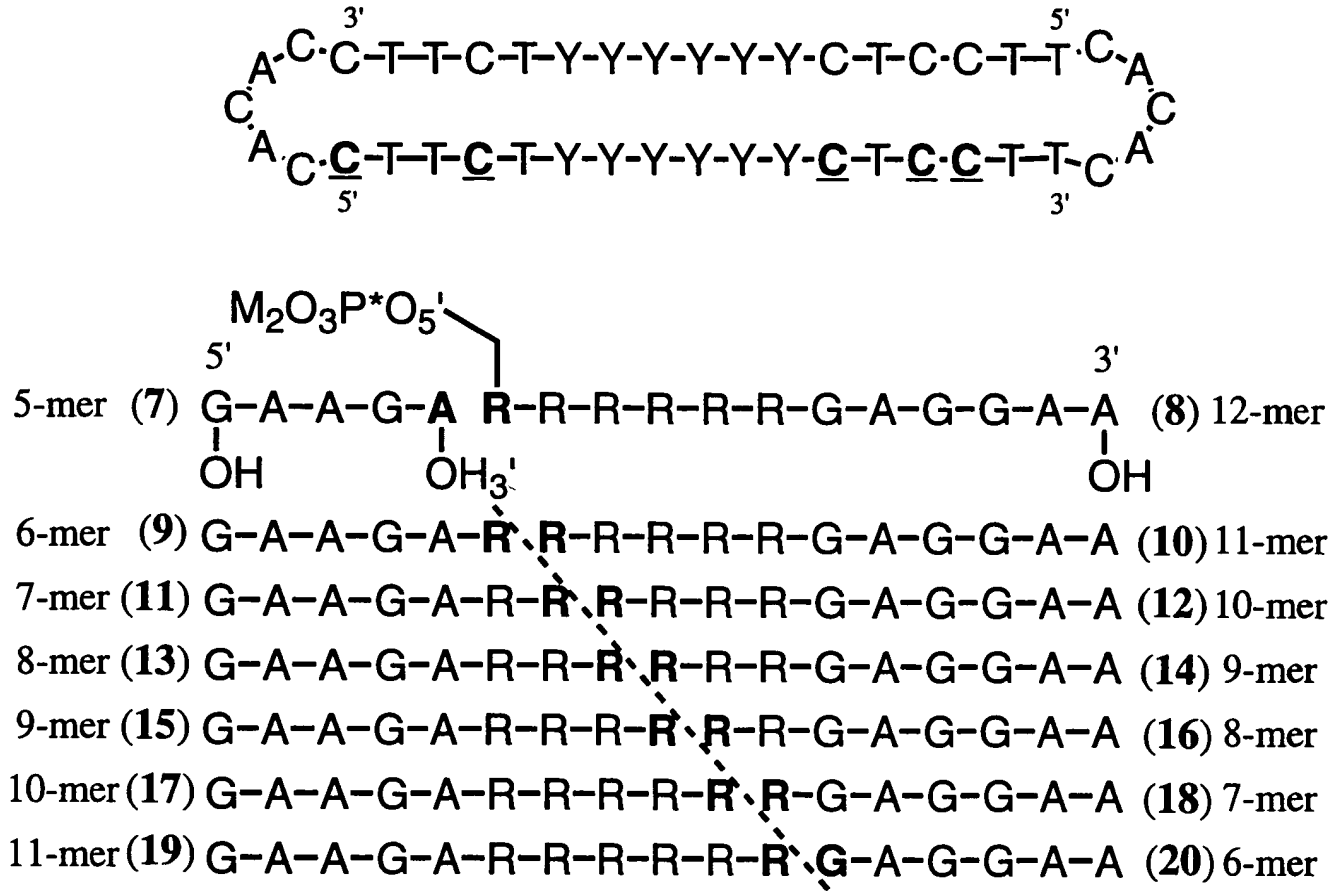


FIG. 11 Ligation analysis investigations in order to probe the effect of Oligonucleotide length and directionality on the ligation reaction. P* represents the P^{32} labelled phosphate which will undergo phosphodiester bond formation.

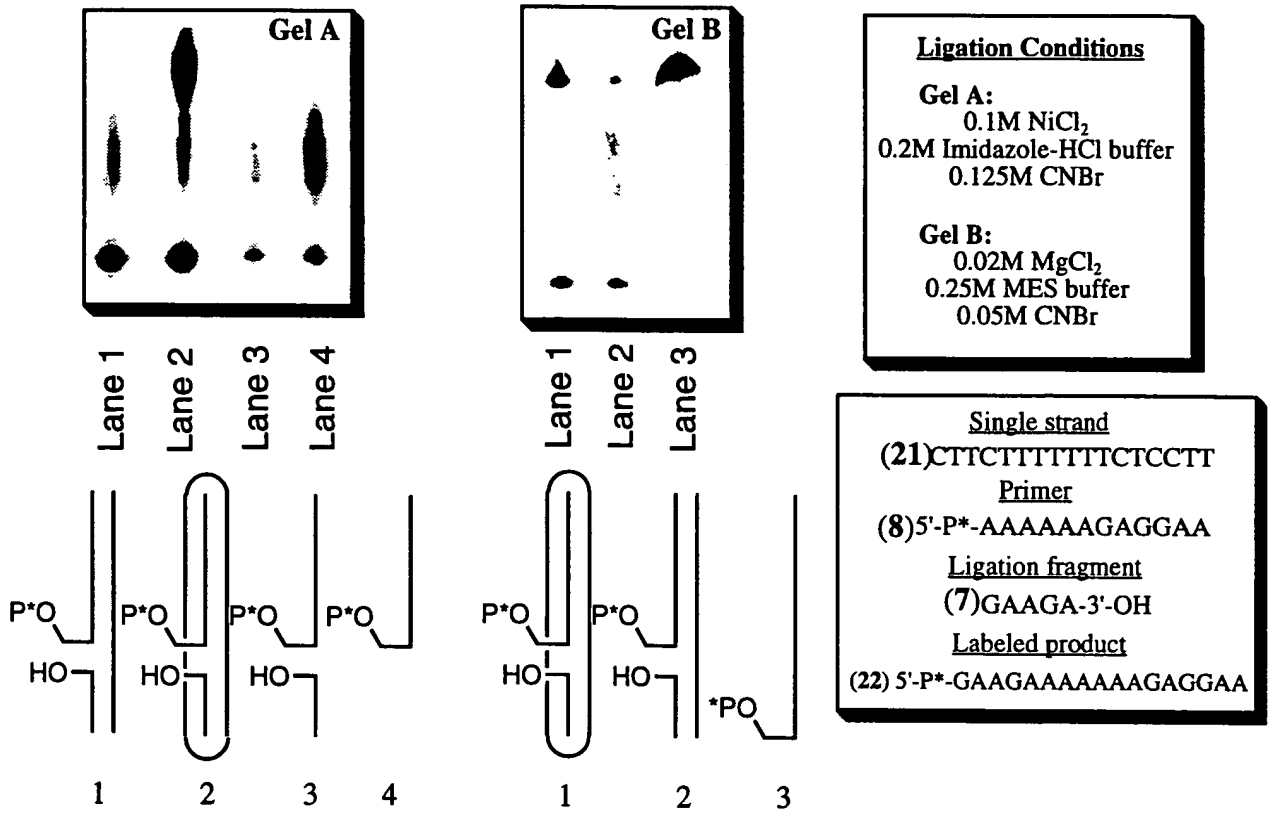
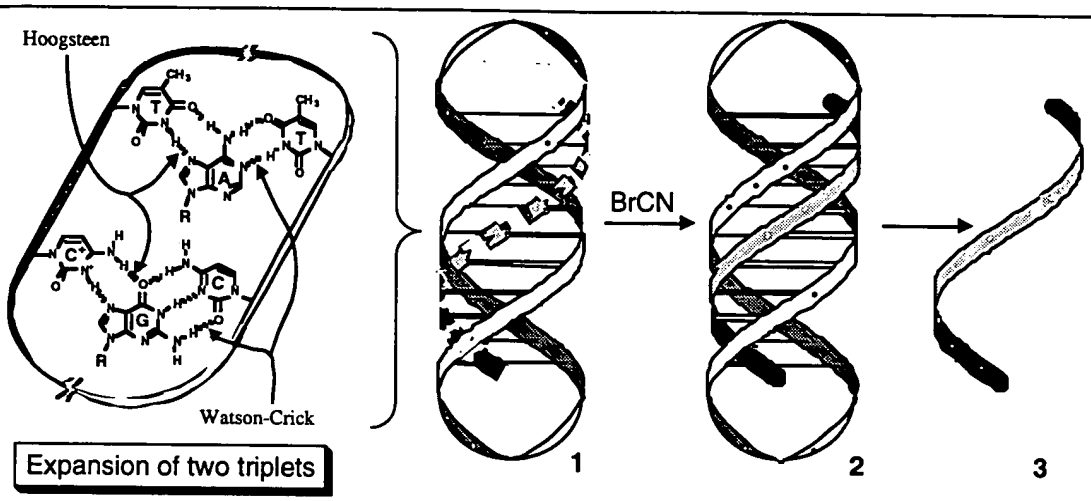
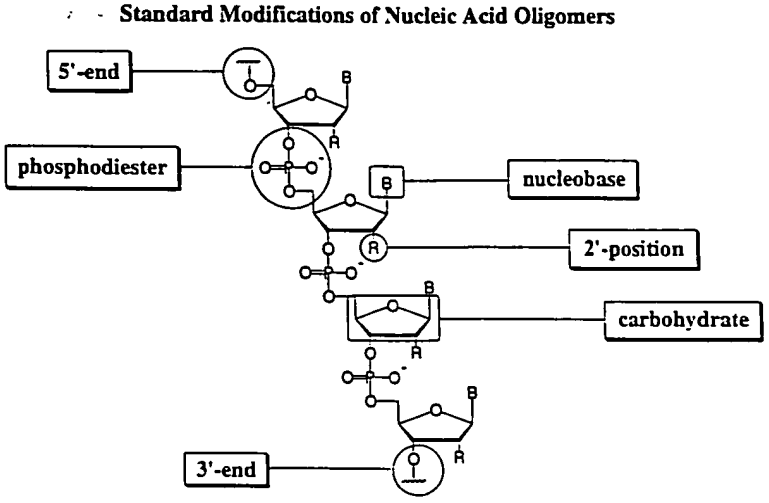


FIG. 12 Autoradiography of PAGE analysis of the ligation of 5-mer **7** with 5'-32 P labeled 12-mer **8**. Gel A shows the results of the reaction run with NiCl2 in imidazole•HCl buffer, while Gel B shows the result of the reaction run with MgCl2 in MES buffer. Lanes 2 (Gel A) and 1 (Gel B) show the migration of the reaction mixture from ligation on circular DNA template **1**. Lanes 1 (Gel A) and 2 (Gel B) compare the results of the ligation reaction on the corresponding single-starnd DNA template **X**. Lane 3 (Gel A) shows the results of the ligation reaction with no template present. Lane 3 (Gel B) shows the migration of the expected full length ligated product which was independently synthesized and 5'-32 P labeled.



The circular DNA template allows hydrogen bonding to both available sites of the monomer-nucleic acid base precursors. The improved thermodynamics greatly increase the efficiency of this template directed oligomerization. [**R** = polymerizing backbone precursor]

Scheme 16



(10) Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1993**, *49*, 1925-63.
(11) Uhlmann, E.; Peyman, A. *Chem Rev.* **1990**, *90*, 543-84.

Figure 13 . Components of nucleic acid polymers that are commonly modified to induce selective properties or functionality to an oligomer.

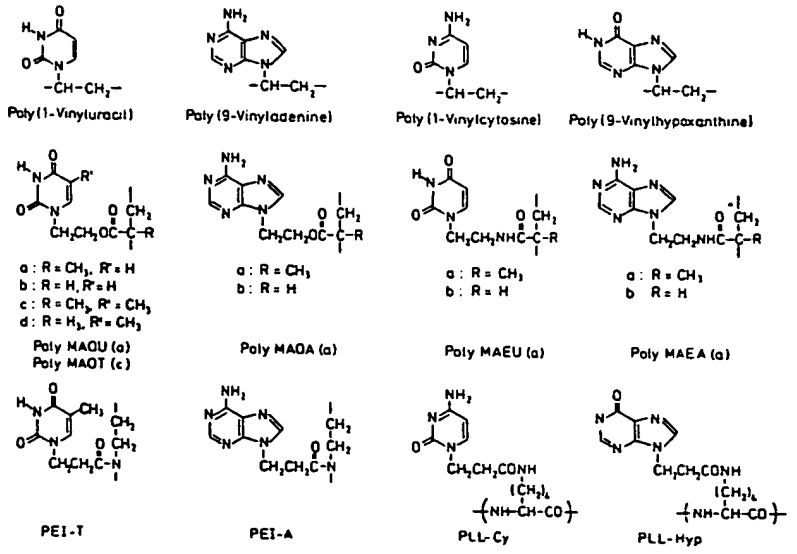


Figure 14. Synthetic Nucleic Acid Analogs.